

What is claimed is:

1. A method for generating a phosphorylatable polypeptide, comprising the steps of:
 - (i) identifying mutation(s) in an internal sequence of a polypeptide necessary to generate a recognition sequence for a kinase;
 - 5 (ii) producing a mutant polypeptide, the sequence for which is identified in step (i); and,
 - (iii) exposing the mutant polypeptide to the kinase and determining the level of phosphorylation of the mutant polypeptide.
- 10 2. The method of claim 1 wherein the mutant polypeptide is recombinantly produced.
3. A method for generating a phosphorylatable polypeptide, comprising the steps of:
 - (i) providing a computer model of a polypeptide of interest;
 - (ii) identifying mutation(s) to an internal sequence of the polypeptide necessary to generate a recognition sequence for a kinase;
 - 15 (iii) using the computer model to determine if the three-dimensional structure and/or biological activity of the mutant polypeptide, either phosphorylated or unphosphorylated at the putative phosphorylation site, will likely be retained, and if so, if the putative phosphorylation site(s) is accessible to the kinase, and if so, if the phosphate group attached to the putative phosphorylation site can be stabilized by intramolecular interactions; and
 - 20 (iv) recombinantly producing a mutant polypeptide if all conditions in step (iii) are met.
4. The method of claim 3, wherein the computer model of the polypeptide of interest is generated using a method selected from any one of the following:
 - 25 (i) X-ray crystallography;
 - (ii) NMR; or,
 - (iii) computer software, using coordinates of a template polypeptide sharing at least 75%, preferably 85%, most preferably 95% sequence homology with

the polypeptide of interest, either over the entire length or at least in the region where mutagenesis is to be carried out.

5. The method of claim 4, wherein the putative phosphorylation site(s) is recognizable by either serine/threonine kinases or tyrosine kinases.
- 5 6. The method of claim 5, wherein the phosphate group attached to the phosphorylated polypeptide is substantially stable so that at least 80%, more preferably 95%, and most preferably 99% of all phosphate groups remain attached after at least 5 days of incubation in animal serum or buffers.
7. The method of claim 6, wherein no more than three amino acid sequences need to
10 be changed to create any single putative phosphorylation site for an intended kinase.
8. The method of claim 6, wherein the polypeptide of interest is an antibody.
9. The method of claim 8, wherein the antibody is a monoclonal antibody.
10. The method of claim 9, wherein the monoclonal antibody is selected from the
15 group consisting of a modified monoclonal antibody, a chimeric antibody, a hybrid antibody, a Fab fragment, a Fab' fragment, and an Fc fragments.
11. The method of claim 8, wherein the antibody is labeled with a radio-isotope.
12. The method of claim 11, wherein the radio-isotope is selected from the group consisting of [³²P], [³³P], [³⁵S] and [³⁸S].
- 20 13. A phosphorylatable polypeptide comprising at least one internal sequence as engineered protein kinase(s) phosphorylation site, which can be stably phosphorylated, whereby the phosphate group attached to the phosphorylated polypeptide is substantially stable so that at least 80%, more preferably 95%, and most preferably 99% of all phosphate groups remain attached after at least 5 days
25 of incubation in animal serum or buffer.
14. The phosphorylatable polypeptide of claim 13, wherein the polypeptide is generated using the method of claim 1.

15. A phosphorylated polypeptide comprising at least one internal sequence as an engineered protein kinase(s) phosphorylation site, which is stably phosphorylated at the phosphorylation site, whereby the phosphate group attached to the phosphorylated polypeptide is substantially stable so that at least 80%, more preferably 95%, and most preferably 99% of all phosphate groups remain attached after at least 5 days of incubation in animal serum or buffer.
16. Polynucleotide sequences encoding polypeptides of claim 13.
17. Polynucleotide sequences encoding polypeptides of claim 14.
18. A kit comprising at least one phosphorylatable polypeptide of claims 13 or 14, or a polynucleotide sequence of claims 16 or 17; at least one protein kinase, or polynucleotide sequence encoding the said protein kinase, capable of phosphorylating the said polypeptide at its engineered phosphorylation site; and at least one nucleic acid or its derivative that is capable of being used as a substrate by the protein kinase to label the phosphorylatable polypeptide.
19. A method to generate computer model(s) of a polypeptide of interest, comprising the steps of :
 - (i) providing the three-dimensional structure and the coordinates of a template molecule that shares at least 75%, preferably 85%, most preferably 95% sequence homology with the polypeptide of interest, at least over the region of the molecule where computer modeling is to be carried out;
 - (ii) developing a molecular model of the polypeptide of interest using a homology modeling program and coordinates of the template molecule, whereby individual subunits of a multi-subunit protein are separately modeled; and
 - (iii) carrying out the geometry refining and energy minimization steps using a molecular modeling software.
20. A method for analyzing the biochemical properties of a polypeptide by using molecular modeling tools, comprising the steps of:

- (i) providing the sequence of a polypeptide of interest and the 3-dimensional structure of the polypeptide, or a model polypeptide of significant sequence homology with the polypeptide of interest;
- (ii) predicting the 3-dimensional structure of the polypeptide of interest or its mutants by computer-aided molecular modeling using the coordinates of the model polypeptide as a template; and
- (iii) determining the energy and stability of the predicted structure of the polypeptide of interest.